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Oral exposure to the organophosphorus insecticide, Monocrotophos induces intestinal dysfunction in rats

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ABSTRACT

There is limited experimental evidence to imply the role of organophosphorus insecticides on intestinal dysfunctions. Residues of Monocrotophos (MCP), above maximum residue limits (MRL), have been reported in fruits and vegetables from various parts of India. Hence, in this study, we investigated the potential of MCP to induce intestinal dysfunction in rats. MCP was administered orally to rats at sublethal doses (0.45, 0.9 and 1.8 mg/kg b.w/d) for 30 days. MCP at the highest dose significantly increased the unit weight of the small intestine. MCP increased the activities of intestinal brush border disaccharidases, intestinal alkaline phosphatase, glycyl-glycine dipeptidase, and Na^+/K^+-ATPase while it decreased cholesterol: phospholipid ratio. Histology and scanning electron microscopy of small intestine of MCP treated rats revealed disruption in terms of congestion, increased length of villi, goblet cell hyperplasia, infiltration of inflammatory cells and necrotic villi tip. Further, the intestinal transit rate was found to be increased in MCP treated rats. Collectively, our findings provide evidence that repeated oral intake of MCP has the propensity to alter small intestinal structure and functions, which might lead to intestinal dysfunctions and abnormal nutrient uptake and thereby affect the human health. Although we have employed doses, which are higher than those likely to be encountered as residues, we speculate that further studies should be performed to determine whether MCP residues in foods in the long-term will interfere with the digestive capacity of the small intestine and thus exert adverse effects on the health of human.

1. Introduction

Organophosphorus insecticides (OPI), currently being used worldwide for insect control were initially introduced as replacements for the persistent organochlorine pesticides. OPI are hydrolyzed in soil or surface water and hence generally not stable (Galloway and Handy, 2003). Perhaps, the instability of these substances is the very reason for their widespread use, which has resulted in the environmental pollution and potential situation of health hazard to non-target organisms. OPI have low environmental persistence and high effectiveness, but overuse and abuse have resulted in residue problems that may cause risks to human health (John et al., 2008). Cholinergic neurotoxicity is essentially the underlying mechanism of acute toxicity of OPI. It is a consequence of inhibition of acetylcholinesterase (AChE), which plays a major role in neurotransmission by hydrolyzing acetylcholine (ACh). In addition, OPI have been shown to be associated with dysfunctions of the reproductive system, metabolic dysregulations, immunotoxicity and oxidative stress. However, very few OPI have been studied with regard to their potential to alter the structure and functions of the small intestine (Chowdhury et al., 1980; Sharma et al., 2011).

The intestine is a major organ of the digestive system and is the primary site of exposure to nutrients/toxicants because of its extensive surface area and physiological properties (Zucco, 1993). The intestinal epithelium constitutes one of the most important cellular membranes because of its role in digestive and absorptive functions. The intestinal mucosa is recognized as a major determinant for the bioavailability of orally administered drugs regulating their absorption, but also biotransformation into metabolites (Suzuki and Sugiyama, 2000). Any alteration in the functional/structural features of the intestine can have a tremendous impact on the overall health of the organisms since this could lead to an inadequate nutrient supply to the organism or result in certain

Abbreviations: ACh, acetylcholine; AChE, acetylcholinesterase; ADI, acceptable daily intake; ALP, alkaline phosphatase; BBE, brush border enzyme; BBM, brush border membrane; MCP, Monocrotophos; MRL, maximum residue limit; OPI, organophosphorus insecticide; PEG, polyethylene glycol ; SEM, scanning electron microscopy; TMDI, Theoretical Maximum Daily Intake.

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disease pathology or aggravate any pre-existing disease pathology. In recent times, gastrointestinal (GI) disorders are on the increasing globally. However, the etiological factors contributing toward most of the disorders of intestine such as, Crohn's disease, irritable bowel syndrome, ulcerative colitis, inflammatory bowel disease and GI bleeding are not clearly understood. Increased stress levels altered food habits and environmental factors are speculated to play major roles (Blumberg and Strober, 2001). Therefore, it is interesting to study the effect of chemicals or contaminants present in food such as pesticides, heavy metals, mycotoxins that reach the gastrointestinal (GI) tract.

Monocrotophos (dimethyl (E)-1-methyl-2-(methylcarbamoyl) vinyl phosphate) (MCP) is a broad spectrum systemic insecticide. As a result of its widespread use, MCP has been detected in ground, surface and rain water (Waite et al., 1992). Monocrotophos is one of the highest consumed pesticides in India. Levels of MCP above maximum residue limits (MRL) (0.2 μg/g) have been detected in vegetables such as brinjal, okra, cauliflower and pea in India (Kumari et al., 2004). According to a recent study conducted in India (Bhushan et al., 2013), the Theoretical Maximum Daily Intakes (TMDI) of MCP was found to be 0.17 mg/d, which was 472% above the Acceptable Daily Intakes (ADI) (0.0006 mg/kg b.w/d or 0.036 mg/d for an adult, FAO/WHO, 1993). Hence, the continuous consumption of vegetables, even with only moderate contamination levels, may lead to varying degrees of toxic effects in the human population after long-term exposures.

Only limited data are available regarding the impact of MCP on tissues/organs in non-target organisms (Czyzewska et al., 1983; Velmurugan et al., 2007). Earlier studies from our laboratory had shown that MCP possesses the potential to alter glucose homeostasis in rats (Joshi and Rajini, 2012), alter lipid profile, intensify the pre-existing disrupted glucose homeostasis and the hepatic/renal oxidative stress among diabetic rats (Begum and Rajini, 2011). Hence, the present work has been carried out to investigate the toxic impact of MCP on small intestine of rats in terms of structure and functions by analyzing the intestinal brush border enzymes activities, lipid composition of brush border membrane and intestinal motility.

2. Materials and methods

2.1. Chemicals

All the chemicals used were of analytical grade and procured from Merck Limited (Mumbai, India) while the standard chemicals were obtained from Sigma Chemicals Co., USA. Technical grade Monocrotophos (78%) was a gift from Hyderabad Chemicals Limited, Hyderabad, India.

2.2. Animals

Adult male rats (CFT – Wistar strain, 8 weeks old, 180 ± 10 g) used for the study were housed in polypropylene cages (2/cage) at room temperature (25 ± 2 °C) with relative humidity of 50–60% and on a 12 h light–darkness cycle. The animals had free access to food and water ad libitum. They were acclimatized to the pelleted diet (Saidurga Feeds and Food, Bangalore, India) for 7 days prior to the start of the experiment. During experimentation, care was taken to minimize animal suffering, and in addition, the number of rats used was kept to a minimum. All procedures with animals were conducted strictly in accordance with guidelines approved by the Institute Animal Ethical Committee, regulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

2.3. Experimental design

Two sets of rats were maintained in each group. Rats were randomly divided (by simple randomization method) into four groups with six animals in each group ($n = 6$). The first group served as the control group. The next three groups were treatment groups to which Monocrotophos (MCP) dissolved in distilled water was given orally at different concentrations (0.45, 0.9, 1.8 mg/kg b.w/d) for 30 days. The doses of MCP selected were 1/40, 1/20 and 1/10th of LD_{50} values based on the reported LD_{50} values (Pesticide Manual, 1999) and our preliminary dose-determination study. Body weight of rats was recorded at regular time intervals during the study. At the end of treatment schedule, intestinal transit was recorded in a set of rats, while the remaining rats were sacrificed by decapitation, and small intestine was excised for biochemical and pathological studies.

2.4. Measurement of intestinal motility

A set of rats (control and treated) received an injection of 1 ml of methylene blue solution (2%) by oral gavage (Anitha et al., 2006). The rats were sacrificed 30 min after receiving the methylene blue solution. The stomach was ligated with a thread above the lower esophageal sphincter and a string beneath the pylorus to prevent leakage of methylene blue. The length of the small intestine was measured by laying out the small intestine on a measuring scale and measuring the distance from the pyloric sphincter to the ileo-cecal valve. The intestinal transit was quantified by measuring the distance from the pylorus to the most distal point of migration of methylene blue and expressed as 'percent intestinal transit' (distance traversed by methylene blue X 100/total intestinal length).

2.5. Tissue processing

Other set of rats (control and treated) were dissected, small intestines were taken out, cut into duodenum, jejunum and ileum. Each segment was flushed with chilled saline (0.9% NaCl). Weight and length of intestine was measured. Jejunum part of the small intestine was selected for histological, scanning electron microscopic and biochemical studies. For the assay of brush border enzymes, intestinal (jejunum) lumen was cut open; mucosa was scraped off using a glass slide. A 10% (w/v) homogenate was prepared in phosphate buffer (100 mM, pH 7.4) and centrifuged for 15 min at 4 °C at 8000 g. The supernatant so obtained was used for various biochemical assays. Whole jejunum was used for the assay of acetylcholinesterase (AChE).

2.6. Acetylcholinesterase (AChE) activity

AChE activity was determined in jejunum homogenate (10% in phosphate buffer 100 mM, pH 7.4) by employing Ellman's method (Ellman et al., 1961), made suitable for microplate reader analysis (Galani and Bocquene, 1991). The assay was performed in 96-well microtiter plate in a total volume of 0.34 ml by the addition of acetylthiocholine iodide (ATCI, the substrate) to a mixture containing suitable amount of tissue homogenate (source of enzyme) and DTNB in phosphate buffer (100 mM, pH 8). Change in absorbance was monitored over 2 min at 405 nm using a microplate reader. The amount of enzyme producing a change of 0.001 units of absorbance per minute was considered as one unit of enzyme and the results were expressed as units/mg protein.

2.7. Brush-border enzyme (BBE) activity

2.7.1. Disaccharidase

Disaccharidase activity was monitored as described by Dahlquist (1964). In brief, suitable amount of homogenate (as enzyme source) was incubated with 56 mM substrates (sucrase, maltase, lactase and trehalase) in 100 mM maleate buffer (pH 6.5) for 10 min at 37 °C. The reaction was stopped by adding 0.5 M Tris, and the released glucose was estimated using the commercial kit (Span Diagnostics Ltd., Mumbai, India) based on the GOD-POD method and results were expressed as nmol of glucose released/min/mg protein.

2.7.2. Dipeptidase

The dipeptidase assay was carried out according to the method of Josefsson and Lindberg (1965). In brief, appropriate volume of homogenate as enzyme source was incubated at 40 °C with 0.1 ml dipeptide solution (glycyl-glycine, glycyl-leucine and glycyl-valine) and 0.2 ml phosphate buffer (0.15 M, pH 7.5). After 10 min, hydrolysis was interrupted by the addition of 2.6 ml ethanol–water (99:1, v/v). The solutions were allowed to stand for 10 min and then centrifuged for 30 min at 3000 rpm. The absorbance of supernatant was measured at 220 nm. Identically treated samples, to which enzyme solutions added after the addition of ethanol–water served as blanks. Results were expressed as μmol of dipeptide hydrolyzed/min/mg protein.

2.7.3. Alkaline phosphatase

Alkaline phosphatase activity was determined using p-nitrophenyl phosphate (PNPP) as substrate (James et al., 1987). Brush border homogenate was incubated with substrate (10 mM PNPP) in Tris buffer (0.05 M, pH 10.1) containing 50 mM $MgCl_2$ at 37 °C for 10 min. After incubation, reaction was stopped by the addition of 0.05 N NaOH. Similarly treated samples, to which homogenate added after the addition of NaOH served as blank. Absorbance was read at 405 nm against respective blanks and results were expressed as nmol p-nitrophenol (PNP) formed/min/mg protein.